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Interaction and Interdependent Packaging of Tegument Protein UL11 and Glycoprotein E of Herpes Simplex Virus▽

Jun Han, Pooja Chadha, David G. Meckes, Jr., Nicholas L. Baird, and John W. Wills*

Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

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The UL11 tegument protein of herpes simplex virus plays a critical role in the secondary envelopment; however, the mechanistic details remain elusive. Here, we report a new function of UL11 in the budding process in which it directs efficient acquisition of glycoprotein E (gE) via a direct interaction. *In vitro* binding assays showed that the interaction required only the first 28, membrane-proximal residues of the cytoplasmic tail of gE, and the C-terminal 26 residues of UL11. A second, weaker binding site was also found in the N-terminal half of UL11. The significance of the gE-UL11 interaction was subsequently investigated with viral deletion mutants. In the absence of the gE tail, virion packaging of UL11, but not other tegument proteins such as VP22 and VP16, was reduced by at least 80%. Reciprocally, wild-type gE packaging was also drastically reduced by about 87% in the absence of UL11, and this defect could be rescued in *trans* by expressing U_L11 at the U_L35 locus. Surprisingly, a mutant that lacks the C-terminal gE-binding site of UL11 packaged nearly normal amounts of gE despite its strong interaction with the gE tail *in vitro*, indicating that the interaction with the UL11 N terminus may be important. Mutagenesis studies of the UL11 N terminus revealed that the association of UL11 with membrane was not required for this function. In contrast, the UL11 acidic cluster motif was found to be critical for gE packaging and was not replaceable with foreign acidic clusters. Together, these results highlight an important role of UL11 in the acquisition of glycoprotein-enriched lipid bilayers, and the findings may also have important implications for the role of UL11 in gE-mediated cell-to-cell spread.

The egress of herpes simplex virus (HSV) from infected cells is generally thought to follow the envelopment/de-envelopment/re-envelopment paradigm (21, 37, 38). The viral capsids are initially assembled in the nucleus of host cells, where they bind particular "inner" tegument proteins, acquire a temporary envelope at the inner nuclear membrane, and then undergo de-envelopment by fusion with the outer nuclear membrane. After the addition of other tegument proteins during capsid transport through cytoplasm, re-envelopment ensues at the *trans*-Golgi network (TGN). There, the final complement of tegument proteins and the glycoproteins occurs during budding, which is mediated by the interaction between tegument proteins and the cytoplasmic tail of viral glycoproteins.

The focus of this study was the HSV UL11 tegument protein, which is a small (96-residue), myristylated and palmitoylated protein and has homologues among all herpesviruses (30, 31). This peripherally bound membrane protein has been shown to play an important role in the re-envelopment process (2, 17, 24, 25) and, consequently, mutants lacking UL11 exhibit a large decrease in virion production and accumulate capsids within the cytoplasm (2, 3, 17). Although the detailed mechanisms remain poorly understood, it is likely that the block in cytoplasmic envelopment is due in part to loss of the UL11-UL16 interaction (27). UL16 is a capsid-associated tegument protein that interacts with an acidic cluster (AC) in the first half of UL11 (36). UL11-null mutants have revealed large defects in packaging of UL16 (3, 35), which is also required for efficient virion production with null-mutants having an ~10-fold reduction in titers (1). Although the membrane-binding and UL16-binding functions reside in the N-terminal half of UL11 (4, 26, 27), the C-terminal half is rather variable among its herpesvirus homologues, and no definitive role has yet been assigned. The data from the present study show that the non-conserved C terminus of UL11 actually also encodes a function to bind to the cytoplasmic tail of gE.

gE is a 552-amino-acid (aa) type I membrane glycoprotein and functions to inhibit complement activation and antibody-dependent cellular cytotoxicity by forming a heterodimer with gI that binds to the Fc portion of antibodies (5, 20, 22, 29, 39). gE also is required for efficient lateral spread of HSV, which has been well documented by numerous studies (9, 10, 43). Consequently, mutants lacking gE are attenuated and display markedly reduced cell-to-cell spread in epithelial and neuronal tissues both *in vitro* and *in vivo* (9–11, 32, 33, 43, 44, 46). The third emerging function of gE is to promote secondary envelopment (6, 7, 15, 16), which works in conjunction with gD and gM. Mutants lacking gE/gD (HSV) or gE/gM (pseudorabies virus) accumulate large aggregates of unenveloped capsids in the cytoplasm (7, 15, 23).

A single report has provided limited data to suggest that UL11 interacts in some manner with gE, as evidenced by co-immunoprecipitation assays from infected cell lysates (16). However, neither the details of the putative interaction nor the significance of the interaction have been elucidated. The experiments described in the present study confirmed the UL11-gE interaction, mapped the interacting sequences, and showed their significance in secondary envelopment. We demonstrate that UL11 interacts with the cytoplasmic tail of gE in

* Corresponding author. Mailing address: Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, 500 University Drive, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-3528. Fax: (717) 531-6522. E-mail: jww4@psu.edu.

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a manner that does not require any other viral proteins or eukaryotic host factors, and this interaction enables efficient, mutual packaging of both UL11 and gE. The determinant for UL11-mediated gE packaging was found to be the acidic cluster motif, which could not be replaced with foreign equivalents from HIV Nef or furin.

MATERIALS AND METHODS

Cells, viruses, and antibodies. Vero cells were maintained in Dulbecco modified Eagle medium (DMEM; Gibco) supplemented with 5% fetal bovine serum (FBS), 5% fetal calf serum, penicillin (65 μg/ml), and streptomycin (131 μg/ml). All viruses were derived from the Kos strain that has been cloned into a bacterial artificial chromosome (BAC) by David Leib's laboratory (18). Mutant viruses including ΔUL11, UL11(Myr-), UL11(Myr-)-GFP, UL11(CCC-), UL11(CCC-)-GFP, and U1 have been previously described (3). For infection assays, Vero cells were grown in DMEM supplemented with 2% FBS, 25 mM HEPES buffer, glutamine (0.3 μg/ml), penicillin, and streptomycin.

The green fluorescent protein (GFP)-specific rabbit serum (diluted 1:4,000) was raised against His_6-GFP and recognizes both GFP and the His_6 tag (4). The UL16 peptide antibody (1:3,000) was raised in rabbits against an N-terminal sequence (RPDSRAGARGTR). Rabbit anti-UL11 antibody was raised against glutathione *S*-transferase (GST)-UL11 and reported before (27). The polyclonal glycoprotein E (gE) antibody was a generous gift from Harvey M. Friedman (University of Pennsylvania) and used at a 1:3,000 dilution. Mouse monoclonal antibody 3114 to gE was kindly provided by David Johnson, and used for immunostaining at 1:4,000 dilution. Mouse monoclonal anti-gD antibody (DL6) was kindly provided by Gary Cohen and Roselyn Eisenberg (University of Pennsylvania) and used at a dilution of 1:15,000. Rabbit antibodies to VP22 (1:3,000), VP5 (1:15,000), and VP16 (1:3,000) were provided by Richard Courtney at the Pennsylvania State University College of Medicine.

Construction of UL11 and gE expression plasmids. The gE cytoplasmic tail was codon optimized and cloned into the vector pGEX-4T-3 at the sites of BamHI and XhoI. For GST-gE.CT derivatives, all of the the constructs used in the present study were created by QuikChange site-directed mutagenesis. The plasmids pUL11-GFP and pUL11(1-50)-GFP were described previously (26). To make pUL11(51-96)-GFP, U_L11 codons 51 to 96 were cloned into vector pEGFP-N2 at the sites XhoI and KpnI in frame with the GFP-coding sequence. The constructs included a Kozak core sequence (GCCACCATGG) for optimal translation. Plasmids pUL11(51-70)-GFP and pUL11(71-96)-GFP were made from pUL11(51-96)-GFP by deletion mutagenesis. pUL11(1-70)-GFP was made by deleting the last 26 codons of U_L11 from pUL11-GFP. To express the corresponding UL11 derivatives in *Escherichia coli*, the alleles were cloned into pET-28a with In-Fusion Advantage PCR cloning Kit (Clontech) to generate the following constructs: pUL11-GFP-His_6, pUL11(1-50)-GFP-His_6, pUL11(51-96)-GFP-His_6, pUL11(50-70)-GFP-His_6, pUL11(71-96)-GFP-His_6, and pGFP-His_6.

Constructs pCMV-gEΔCT and pCVM-gE.CT28 were generated from a eukaryotic expression plasmid pCMV-gE (12) by introducing stop codons after codons for Trp-446 and Asp-470 (respectively) with PCR-based site-directed mutagenesis. pCMV-Src.gE.CT.HA was created by cloning the sequence encoding the gE tail into pEGFP-N2 vector at the sites of HindIII and BamHI. The gE tail was tagged with the N-terminal 10-aa sequence (GSSKSKPKDS) from the v-Src oncogene (47) and a C-terminal hemagglutinin (HA) epitope (YPYDVP DYA). A Kozak core sequence was also included to allow optimal expression.

Purification of GST or His-tagged fusion proteins. Bacterial strain BL21 codon plus containing plasmids for the GST fusion or His-tagged proteins were cultured overnight at 37°C and then inoculated at 1:100 into 100 ml of yeast extract-tryptone medium culture (2×YT). Protein expression was induced for 3 h by addition of IPTG (isopropyl-β-D-thiogalactopyranoside) at a final concentration of 0.1 mM when the optical density at 600 nm reached 0.6 to 0.8 at 37°C. The cultures were then pelleted down at 8,000 rpm for 2 min at 4°C. The cell pellets were suspended in 9 ml of phosphate-buffered saline (PBS) supplemented with bacterial protease inhibitors (P3584′ Sigma), sonicated, and lysed for 30 min on ice with 1% Triton X-100. The lysate was cleared twice at 11,500 rpm for 10 min each time at 4°C, and the supernatants were incubated with glutathione-Sepharose 4B beads (GE Healthcare) or nickel beads at room temperature for 1 h. For GST fusion proteins, the beads were pelleted at 1,000 rpm for 1 min, washed three times with PBS for 10 min each, and then suspended in 300 to 400 μl of PBS. For His-tagged proteins, the beads were washed once with PBS, once with binding buffer, and once with wash buffer. The proteins were eluted with 1 ml of elution buffer at room temperature for 3 h. Yields were determined by SDS-PAGE, followed by Coomassie blue staining.

GST pulldown assays. To analyze the interaction of UL11 with gE, Vero cells were transfected by means of Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Briefly, solutions of Lipofectamine 2000 (10 μl) and 5 μg of plasmid diluted in 1 ml of Opti-MEM (Invitrogen) were applied to Vero cell monolayers in 60-mm petri dishes. The transfection medium was removed after 4 h of incubation and replaced with fresh culture medium (1× DMEM with 10% FBS). At 18 to 24 h posttransfection, the cells were harvested in NP-40 lysis buffer (0.5% NP-40, 150 mM NaCl, 50 mM Tris-HCl [pH 8.0], protease inhibitor cocktail [P8340; Sigma]), precleared with glutathione-Sepharose 4B beads for 2 h at room temperature, and then incubated for 5 h at room temperature with wild-type or mutant GST-gE.CT proteins immobilized on glutathione-Sepharose beads. The beads were washed three times with NP-40 buffer for 10 min each and boiled for 5 min, and the proteins bound to the beads were separated by SDS-PAGE, transferred to nitrocellulose membranes, probed with proper antibodies, and developed by ELC Western blotting system (Pierce).

***In vitro* binding assay.** The *in vitro* binding assay was described previously (49). Briefly, to determine whether UL11 and gE.CT and their derivatives have the ability to interact directly, the purified GST-gE.CT and His_6-UL11 derivatives were incubated in 0.5% NP-40 lysis buffer for 5 h. The proteins bound to the beads were washed three times with NP-40 buffer, suspended in 20-μl sample buffer, boiled for 5 min, transferred to nitrocellulose, Ponceau S stained, and then analyzed by immunoblotting with the anti-His_6-GFP antibody.

Confocal microscopy. Vero cells grown on coverslips in 35-mm petri dishes at 50 to 80% confluence were transfected with UL11-GFP and gE or its truncation mutants. Single transfections of each construct served as control. The cells were fixed with 3.7% paraformaldehyde for 7 min, permeabilized for 15 min with PBS containing 0.1% Triton-100 and 2% bovine serum albumin (BSA), and then blocked with 2% BSA-PBS for 30 min. gE was stained with mouse monoclonal antibody 3114 for 1 h at room temperature in a humid chamber, and washed three times with PBS for 5 min each time. After incubation with secondary antibody (Alexa 568-goat anti-mouse IgG) for another hour, the cells were washed three times with PBS. Nuclear DNA was stained with DAPI (4′,6′-diamidino-2-phenylindole; Molecular Probes). The images were collected under a Leica SP2 confocal microscope.

Construction of recombinant HSV UL11 and gE mutants. A BAC containing the HSV-1 KOS strain genome was used and the detailed protocol to generate a recombinant virus was described previously (3). Briefly, a *galK* expression cassette was used to replace the target gene in the KOS BAC. Next, the *galK* cassette was replaced with a DNA fragment. Correct clones were verified by HindIII digestion, PCR analysis, and DNA sequencing of the corresponding region. The resulting BAC plasmids were purified and then transfected into Vero cells with Lipofectamine 2000. After 3 to 4 days, the transfected cells were harvested when showing cytopathic effects and used to infect new Vero cell monolayers to produce a viral stock.

Viral growth assays. Six-well plates of Vero cells were infected with the specified viruses at a multiplicity of infection (MOI) of 0.1. After 1 h of incubation at 37°C, the cells were first washed with acid buffer (135 mM NaCl, 10 mM KCl, 40 mM citric acid [pH 3.0]), then washed once with DMEM, and finally overlaid with 1 ml of DMEM containing 2% FBS. At the indicated times postinfection, the medium and cells from each well were harvested and treated as follows. The medium was cleared of cells by centrifugation at 12,000 rpm for 1 min and frozen at −80°C. The cells were scraped into PBS, washed three times with PBS, and freeze-thawed three times to release cell-associated viruses. Each sample was titrated on Vero cells by using a standard viral plaque assay.

Virion incorporation assay. Vero cells were infected with wild-type HSV or UL11 or gE mutants at an MOI of 5. At 16 to 24 h postinfection, the medium was collected, cleared at 2,500 rpm for 10 min, and then centrifuged at 26,000 rpm for 1 h at 4°C in a Beckman SW41 rotor through a 30% (wt/vol) sucrose cushion (1.7 ml). The pellets were dissolved in 100 μl of 1× SDS-PAGE sample buffer, and equal quantities of virus were loaded into an SDS-PAGE gel for Western blot analyses.

RESULTS

Can GST-gE.CT pull down UL11 from transfected cells? The initial evidence for UL11-gE interaction stemmed from the coimmunoprecipitation experiments in which the UL11 tegument protein was coimmunoprecipitated with gE from HSV-infected cells (16), suggesting that UL11 and gE are in a complex of some sort. However, it is not clear whether any other viral proteins are required for this interaction or whether

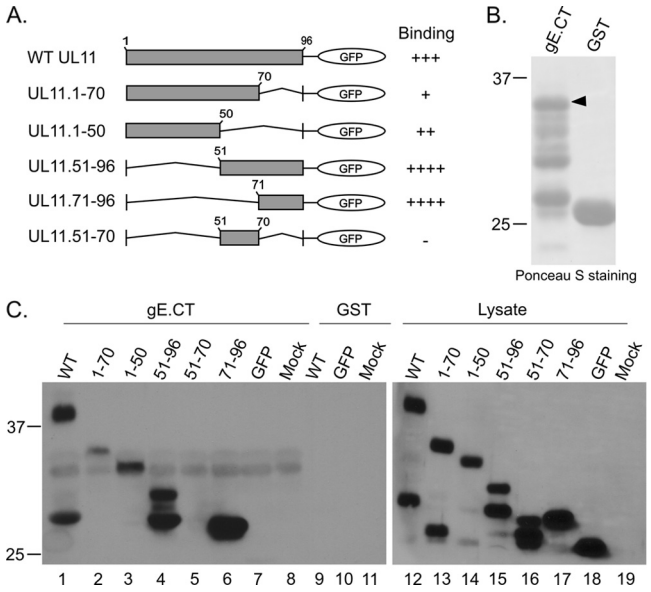

FIG. 1. Mapping of the gE-binding region within UL11. (A) Diagrams of UL11-GFP and a panel of deletion mutants that were used for GST-pulldown experiments. (B) GST and GST fused to the cytoplasmic tail of gE were expressed and purified from *E. coli*, followed by SDS-PAGE and Ponceau S staining. (C) Purified GST-gE.CT or GST-only were used in attempts to pull down full-length UL11-GFP and mutants from transfected Vero cell lysates. Proteins bound to the beads were separated by SDS-PAGE, transferred to nitrocellulose membrane, and subjected to Western blotting with rabbit anti-GFP antibodies (left panel). The right panel shows the expression level of UL11-GFP and its derivatives in Vero cells. A summary of the results is shown in panel A. WT, wild type.

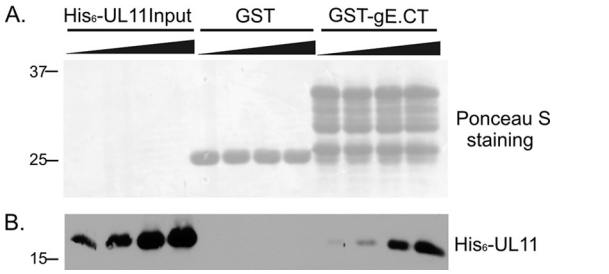

FIG. 2. Direct binding of UL11 to the cytoplasmic tail of gE. UL11 was expressed as a His_6-tagged fusion protein. Purified GST-gE.CT or GST-only were incubated with 2-fold increasing amounts of purified His_6-UL11 in NP-40 lysis buffer at room temperature for 5 h. Proteins bound to GST beads were washed with NP-40 buffer, separated by SDS-PAGE, transferred to nitrocellulose membrane, visualized by Ponceau S staining (A), and probed with rabbit anti-His_6 antibodies (B).

the interaction was specific and not an artifact of working with a "sticky" protein due to hydrophobic fatty acids (16). To test the hypothesis, GST pulldown experiments were performed. The gE cytoplasmic tail (gE.CT) was cloned, expressed as a GST fusion protein, and purified with glutathione beads. Despite codon optimization, the gE tail is heavily degraded (Fig. 1B), which is consistent with observations from other studies (11, 45). Nevertheless, the gE tail was able to efficiently pull down UL11-GFP (Fig. 1A, lane 1) from transfected Vero cell lysates (Fig. 1C, lane 12). In contrast, the GST protein alone did not (Fig. 1C, lanes 10 and 11). In addition to the full-length UL11-GFP, a fast-moving band was also pulled down by GST-gE.CT (Fig. 1C, lane 1). Because this product reacted with GFP antibodies but was bigger than GFP (lane 18), it must contain some portion of the C terminus of UL11, and possibly a gE-binding site. Further, both bands were preexisting in lysates (Fig. 1C, lane 12); hence, this was not degradation that occurred after binding to the gE tail. Nevertheless, it is clear that an interaction between UL11 and the gE tail can take place *in vitro*, and this interaction does not require any other viral proteins.

Is the interaction between UL11 and gE.CT direct? To address this question, His_6-tagged recombinant UL11 was made and purified from *E. coli* as described previously (49). Various amounts of His_6-UL11 were incubated with GST-gE.CT beads in NP-40 buffer for 5 h. The GST protein served as a negative control. UL11 was detected with antiserum that recognizes the His_6 tag. As expected, GST alone did not bind to UL11 protein (Fig. 2); however, GST-gE.CT was able to pull down His_6-UL11 in a dose-dependent manner (Fig. 2). These data indicate that UL11 directly interacts with the gE tail *in vitro*, and no specific eukaryotic host factor is required for this interaction.

Which parts of UL11 bind to the gE tail? The N-terminal half of UL11 has been shown to strongly interact with UL16 (27, 49). To examine whether the same region also is important for binding to gE.CT, a transfection/pulldown assay was performed by using the construct encoding UL11(1-50)-GFP (26) (Fig. 1A). The results revealed that UL11(1-50)-GFP was able to bind to gE.CT (Fig. 1C, lane 3). However, the interaction appeared to be weak since only a small amount of UL11(1-50)-GFP was pulled down from cell lysates even though it was well expressed, suggesting that the C terminus likely contains the major gE-binding region. This speculation is reasonable since the aforementioned fast-moving band containing a portion of the UL11 C terminus was concurrently pulled down with UL11-GFP by the gE tail. To test this hypothesis, the C-terminal half of UL11 [UL11(51-96)] was fused to the N terminus of EGFP (Fig. 1A) and expressed in Vero cells. As hoped, UL11(51-96)-GFP, which also exhibited partial degradation like UL11-GFP, was readily and efficiently pulled down by GST-gE.CT (Fig. 1C, lane 4). Thus, the nonconserved C-terminal half of UL11, which has not been assigned a function before, confers the major binding to the cytoplasmic tail of gE, at least *in vitro*.

In an attempt to pinpoint the critical residues in the C-terminal half of UL11 responsible for gE.CT interaction, site-directed mutagenesis was performed because of the small size of the region. Twenty-one mutants (R53A, T56A, R57A, R61A, Q62A, R63A, R65A, S67A, SDP67RRA, D68A, DPP68RAR, R72A, H73A, T74A, H75A, R77A, T78A, T86A, Q87A, F88A, and P90A) were created and analyzed, but no obvious disruptive effect on UL11-gE interaction was observed (data not shown). This prompted us to switch back to the deletion mutagenesis approach. The mutants were made as shown in Fig. 1A, and their binding abilities were tested in the GST pulldown assay. The results demonstrated that residues 71 to 96, but not 51 to 70, strongly interacted with the gE tail (Fig. 1C, lane 6). This result was also confirmed by the *in vitro*

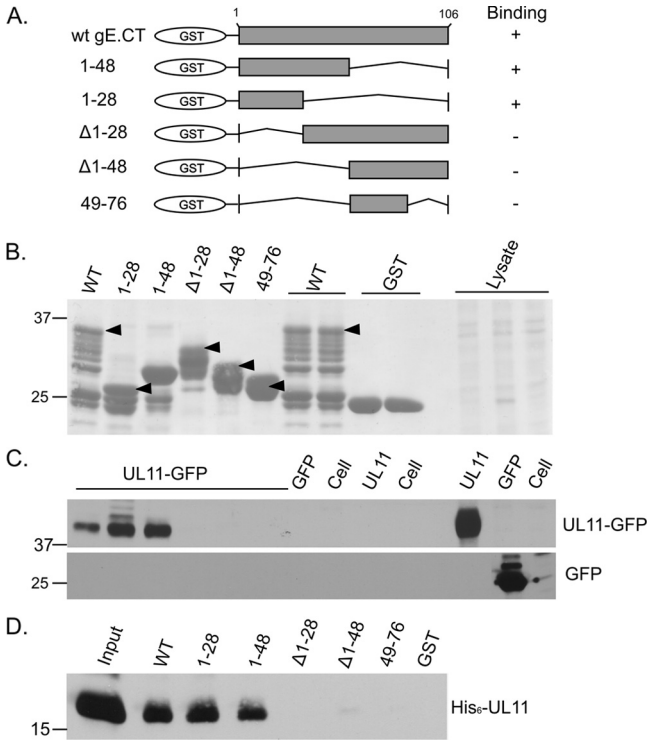

FIG. 3. Mapping of the UL11-binding region within the cytoplasmic tail of gE. (A) A series of gE-tail deletions were constructed as GST fusion proteins. (B) The recombinants were expressed and purified from *E. coli*. Vero cells transfected with UL11-GFP or GFP-only were lysed and incubated with the purified GST or GST-gE.CT proteins described above. Bound proteins were separated by SDS-PAGE, transferred to nitrocellulose membrane, and visualized by Ponceau S staining. (C) Detection of UL11-GFP with rabbit anti-GFP antibodies. (D) The purified GST fusion proteins were also tested for their ability to bind to purified His_6-UL11. A summary of the binding results is shown in panel A.

binding assay with the corresponding His_6-UL11-GFP mutants purified from *E. coli* (data not shown).

Which part of the gE tail binds to UL11? To identify the UL11-binding site within the gE tail, a series of truncation mutants were made from GST-gE.CT (Fig. 3A). The recombinant proteins (Fig. 3B) were purified from *E. coli* and used in attempts to pull down UL11-GFP from transfected Vero cell lysates. As shown in Fig. 3C, the first 28 aa, but not gE.CTΔ1-28, were found to be sufficient for the interaction (Fig. 3C). This result was further confirmed by the *in vitro* binding assay (Fig. 3D).

Do UL11 and gE interact in transfected cells? To test whether UL11 and gE can actually interact within mammalian cells, cotransfection experiments were carried out. When UL11-GFP was expressed alone, it was strongly perinuclear localized (Fig. 4B), and no obvious nuclear membrane staining was observed. In contrast, gE expressed by itself was on the nuclear membrane with speckles throughout the cytoplasm and in the perinuclear region (Fig. 4B). When the two proteins were coexpressed, UL11 appeared to reorganize gE to be more perinuclear, while UL11 was also colocalized with gE in speckled structures in the cytoplasm (Fig. 4C). To ascertain whether the gE tail mediates the interaction, two mutants were constructed: gEΔCT and Src.gE.CT.HA (Fig. 4A). The gE tail truncation mutant was created by adding a stop codon right after the gE transmembrane domain. Unfortunately, this mutant was expressed poorly in Vero cells, and thus we could not assess the colocalization relationship (data not shown). For Src.gE.CT.HA, gE.CT has a HA tag on the C terminus of the gE tail sequence, and a 10-aa membrane-binding sequence from the Src oncoprotein on its N terminus to provide a membrane anchor. When expressed by itself, Src.gE.CT.HA did not appear on the nuclear membrane but showed a dispersed pattern in the cytoplasm (Fig. 4B). When coexpressed, UL11-GFP was well colocalized with Src.gE.CT.HA in speckled structures in the cytoplasm (Fig. 4C), suggesting the gE tail alone can interact with UL11 *in vivo*.

The *in vitro* experiments described above demonstrated that the first 28 aa of the gE tail are sufficient to interact with UL11. To test whether the same is true in *vivo*, we generated a gE truncation mutant that retains the first 28 aa of the gE tail (Fig. 4A). When expressed alone, gEΔCT28 exhibited an ER-like pattern extending outward from, but concentrated more around and on, the nuclear membrane (Fig. 4B). In contrast, when coexpressed with UL11-GFP, the distribution was dramatically changed for both proteins, resulting in a perfect colocalization relationship (Fig. 4C). Thus, the UL11-gE interaction can also occur within cells.

Is UL11 needed for incorporation of gE into virions? This question was addressed to test the relevance of the UL11-gE tail interaction. The UL11-null mutant ΔUL11 (Fig. 5) was described before (3) and exhibited about a 2-log reduction in infectious virus yield compared to wild-type HSV at an MOI of 0.1 (Fig. 6A). Vero cells were infected with the wild-type or ΔUL11 strains at an MOI of 5, and the virions from extracellular medium at 24 h postinfection were pelleted through a 30% sucrose cushion and analyzed. The infected cells were also harvested to analyze the intracellular expression level of various proteins. The sample volumes were normalized based on the level of VP5 and analyzed by immunoblotting. Deletion of UL11 dramatically reduced the packaging level of gE (Fig. 7A), and quantitative analysis revealed a reduction of more than 80% (Fig. 7B). The decreased packaging efficiency was not due to the reduced intracellular expression since the expression levels were comparable (Fig. 7A). To test whether this phenotype is specific to gE, we examined the packaging of gD, which also has been reported to interact with UL11 in some manner (16). In contrast to gE, the packaging level of gD was only reduced by ~20 to 30% (Fig. 7). Since there is a possibility that high MOI infection at 24 h postinfection may result in release of a substantial number of capsids into media that may confound the experiment results, we also tested other time points (16 and 20 h) for virion incorporation analysis and the results were consistent (data not shown). To test the possibility that the effect on gE was due to unintended mutations in the virus genome, we analyzed a revertant construct, U1 (Fig. 5), in which U_L11 was reinserted into the U_L35 locus of HSV ΔUL11 (3), and this restored the packaging level of gE (Fig. 7). Thus, we conclude that the observed phenotype is specific to gE, and UL11 is required for efficient virion incorporation of gE.

Is the packaging of UL11 dependent on the cytoplasmic tail of gE? To test whether the absence of gE has a mutual effect on UL11 packaging, we constructed a gE cytoplasmic tail de-

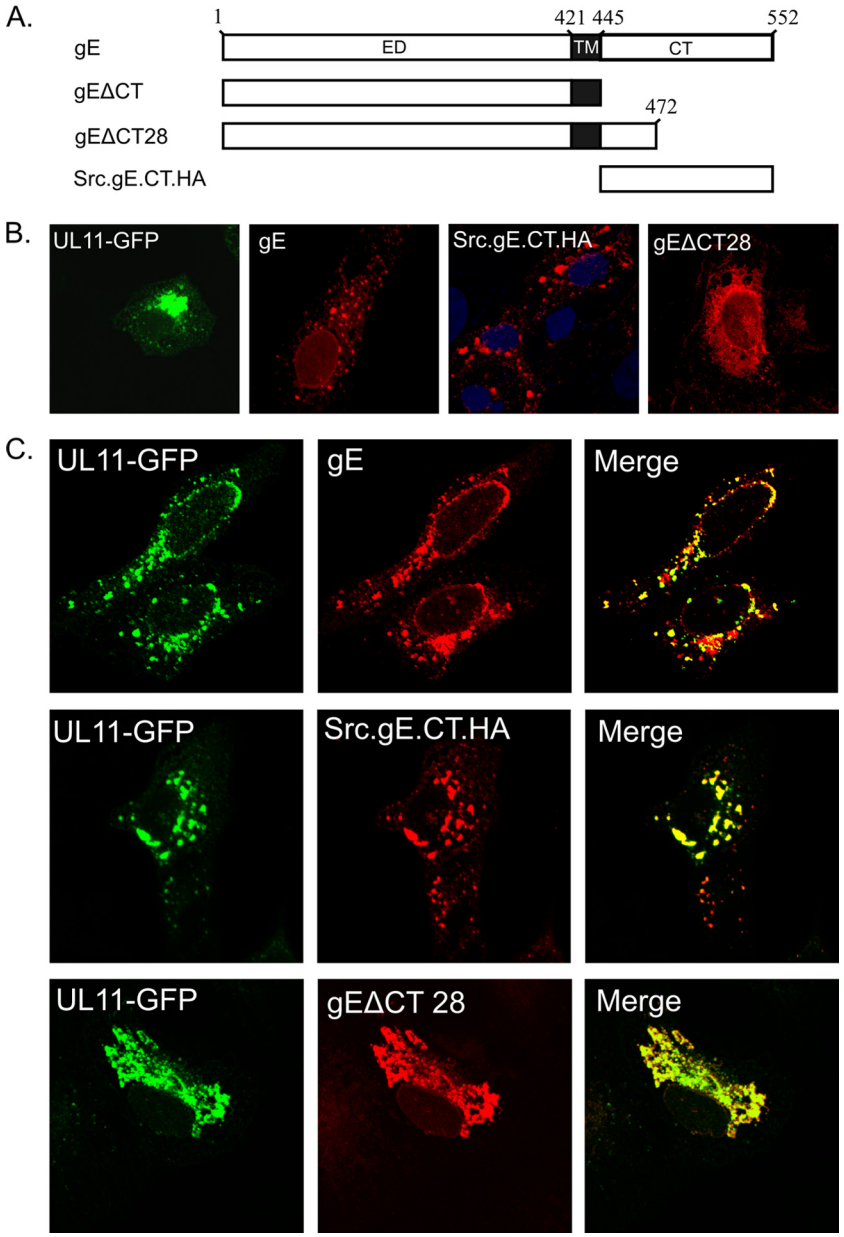

FIG. 4. Colocalization analysis of UL11 and gE. (A) Summary of the gE constructs used in the present study. Vero cells grown on coverslips were transfected with plasmids that express gE constructs alone (B) or cotransfected with a plasmid that expresses UL11-GFP (C). At 16 to 18 h posttransfection, the cells were fixed and permeabilized prior to staining with a gE mouse monoclonal antibody or a monoclonal antibody specific to the HA epitope. The primary antibodies were located with a secondary goat anti-mouse IgG antibody conjugated to Alexa 568. Nuclei were stained with DAPI (blue). The cells were viewed by confocal microscopy.

letion mutant (gEΔCT) by adding a stop codon between those for residues W_{446} and R_{447}, right after the transmembrane domain. The gEΔCT mutant exhibited an ~1-log decrease in production of infectious virions at an MOI of 0.1 (Fig. 6A) and, as expected, a faster-moving form of gE was detected in both virus-infected cells and virions (Fig. 7A), but the amounts were very low, suggesting that gEΔCT is unstable in infected cells, as it was in transfected cells (see above). We then analyzed the gEΔCT virus for the packaging of UL11. Because UL11 contains the signals necessary for membrane binding and trafficking to the TGN (26), where budding takes place and it encounters UL16 and other binding partners, it seemed unlikely UL11 would also need to bind gE to be incorporated. Quite surprisingly, the packaging efficiency of UL11 was severely reduced (>80%) in the absence of the gE tail (Fig. 7), even though the intracellular expression level of UL11 was roughly equal to that of wild-type virus (Fig. 7A).

To test whether the packaging defect is specific to UL11, we examined two other proteins: VP16 and VP22. VP16 is bound to capsid and does not bind to gE but is a binding partner of VP22 (14). As expected, deletion of the gE tail did not affect VP16 packaging (Fig. 7). VP22 is one of the most abundant

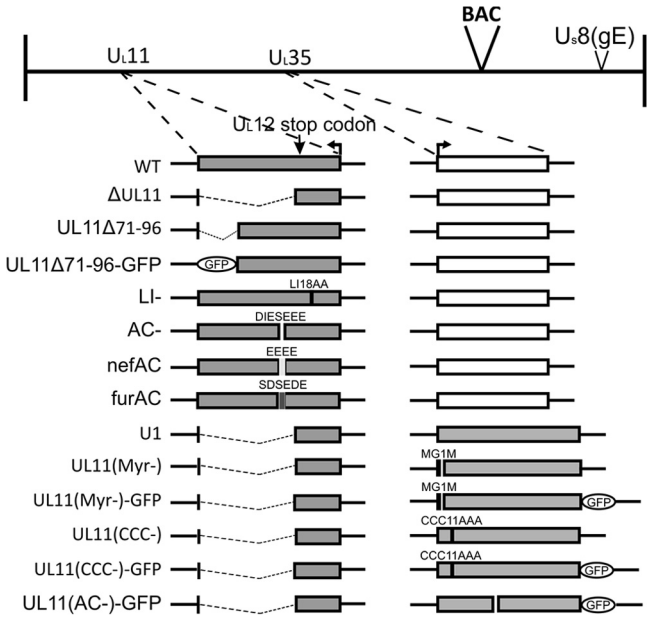

FIG. 5. Mutant viruses used in the present study. A gE-tail truncation mutant was constructed by inserting a stop codon in U_S8 immediately after the coding sequence for the transmembrane domain. The UL11-null mutant (ΔUL11) was created by deleting the codons 31 to 96 but without altering the overlapping and essential U_L12 gene. The mutants U1, UL11(Myr-), UL11(Myr-)-GFP, UL11(CCC-), UL11(CCC-)-GFP, and UL11(AC-)-GFP were constructed independently from the mutant ΔUL11 by replacing U_L35 with the respective UL11 alleles. UL11 mutants LI-, AC-, nefAC, and furAC were made by deletions or substitutions as shown in the diagrams. UL11Δ71-96 was constructed by removing codons 71 to 96 and, similarly, replacement of codons 71 to 96 with the GFP-coding sequence was to construct UL11Δ71-96-GFP. The relative location of the BAC sequence is indicated.

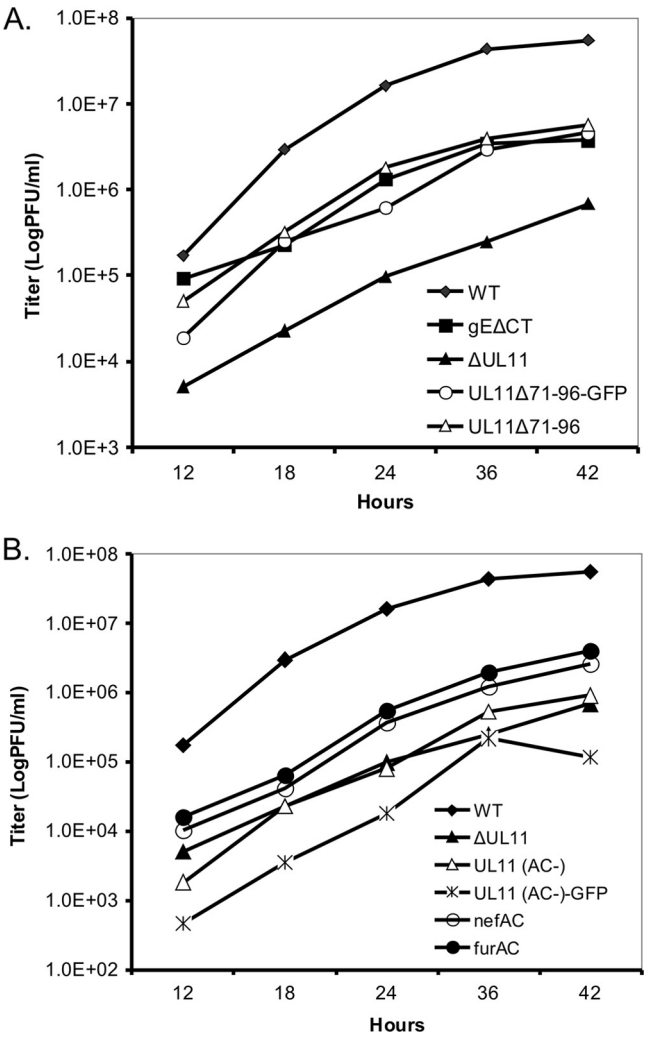

FIG. 6. Multistep growth curves of selected UL11 mutants. Vero cells were infected with the specified viruses at an MOI of 0.1 for 1 h at 37°C and then washed with citrate buffer to inactivate the viruses remaining on the cell surfaces. After being washed with DMEM, the cells were overlaid with 1 ml of DMEM containing 2% FBS. At the indicated times postinfection, media and cells from each well were harvested and analyzed by plaque assay to determine the intracellular and extracellular viral yields, which were added together to produce these graphs. (A) UL11 and gE-tail deletion viruses. (B) Acidic cluster mutants.

tegument proteins in the tegument, and has been shown to interact with the cytoplasmic tail of gE (16, 41). Also, it has been reported that VP22 is recruited to the membranes in a gE-independent manner (8). Hence, it seemed possible that packaging of VP22 would not be dependent on gE, and that is what we found for the mutant gEΔCT (Fig. 7). This finding is also consistent with a recent report that demonstrated that gE is not required for VP22 packaging as measured in a transfection/infection assay (40). Thus, we conclude that the gE tail is required for efficient UL11 incorporation.

Is the C-terminal binding site in UL11 required for gE packaging? UL11 residues 71 to 96 exhibited a strong interaction with the gE tail *in vitro*. To ascertain whether this region is responsible for the crippled gE packaging observed for the UL11-null virus, a recombinant lacking these residues was made (Fig. 5), which possessed intermediate growth kinetics compared to the wild-type and UL11-null viruses (Fig. 6A). This mutant was examined for gE packaging, which was found to be reduced by only 20 to 30% (Fig. 8C). This intermediate level is reasonable to some extent considering the fact that UL11 appears to contain two gE-binding regions. However, this could also be explained by the reduced stability of the truncation product since it was not detectable in either virion particles or infected cells (Fig. 8A and B). To further clarify the role of residues 71 to 96, we generated a derivative of this mutant that has GFP linked to the C terminus immediately after residue 70 (Fig. 5). As hoped, the truncation product UL11(1-70) was stabilized and readily detected in both infected cells and virion particles (Fig. 8A and B). Again, we analyzed the gE packaging level and were surprised to find that it was completely restored to the wild-type level (Fig. 8C), suggesting that UL11(71-96) is not essential for efficient packaging of gE despite its strong interaction with the gE tail *in vitro*. Thus, these results suggest that, in the absence of the UL11 C terminus, gE packaging may be recruited through the N terminus of UL11, which was also capable of interacting with the gE tail (Fig. 1C).

Which part of the N-terminal half of UL11 is needed for gE packaging? Rather than deleting the entire first half of UL11,

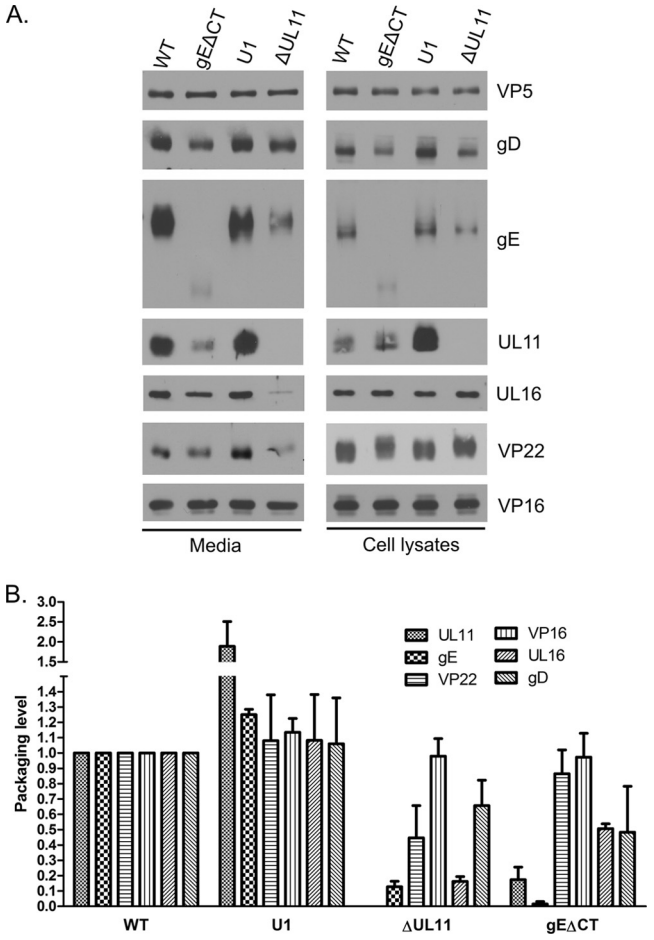

FIG. 7. Interdependent packaging of UL11 and gE into virions. (A) Analysis of the virion compositions of wild type, gEΔCT, U1, and ΔUL11. Extracellular virions were purified from infected cell media, and virion proteins were separated by SDS-PAGE and probed with antibodies against VP5, gE, gD, VP22, UL16, and UL11 (left panels). The infected cell lysates were also analyzed for the expression levels of these proteins (right panels). (B) The amounts of gE, gD, VP16, VP22, UL16, and UL11 in the mutant virions were quantified and normalized to the level of the respective proteins in the wild-type virions. The data represent at least three independent experiments. WT, wild type.

we instead examined the contributions of specific sequence motifs in this region that are required for its known functions of membrane binding, trafficking, and binding to tegument protein UL16 (4, 26). Four mutant viruses [UL11(Myr-), UL11(CCC-), UL11(AC-), and UL11(LI-)] were investigated (Fig. 5). Mutant UL11(Myr-) lacks the N-terminal glycine that is normally myristoylated to provide weak membrane binding, which in turn enables subsequent palmitoylation of nearby cysteines (4). Thus, the UL11(Myr-) protein lacks both fatty acids and is not capable of binding membranes (4). Mutant UL11(CCC-) lacks the cysteines that are normally palmitoylated to provide stable membrane binding and enable entry into lipid rafts (4). This mutant weakly binds membranes because it retains the site of myristoylation (4). Mutant UL11(AC-) lacks the acidic cluster, which is needed for trafficking out of lipid rafts (4) and is also the binding site for UL16 (27), a tegument protein that also binds to capsids and tegument protein UL21 (19, 36). Mutant UL11(LI-) lacks another motif that is needed for trafficking out of lipid rafts (26). As for growth properties, UL11(Myr-) and UL11(CCC-) replicated slightly better than the UL11-null mutants and have been previously described (3). UL11(LI-) showed slight decrease in virion production and formed plaques similar in size to the wild-type virus (data not shown). In contrast, UL11(AC-) behaved just like UL11-null mutant and exhibited indistinguishable growth kinetics (Fig. 6B).

Analyses of infected cells and progeny virions revealed only one mutant that behaved like the wild type, UL11(LI-). It expressed normal amounts of UL11 (Fig. 8B) and packaged normal amounts of gE (Fig. 8A and C). Unexpectedly, mutant UL11(CCC-) also packaged normal amounts of gE (Fig. 8A and C), even though its UL11 derivative could not be detected in cell lysates or virions (Fig. 8A and B). This suggests that only a small amount of UL11 is needed for helping gE to get packaged, and entry into lipid rafts is not a requirement. In contrast, mutants UL11(Myr-) and UL11(AC-) were severely impaired for gE packaging (Fig. 8A and C); however, in both of these cases, expression of the UL11 derivative was very low or not detectable (Fig. 8B). In an attempt to increase stability, GFP-tagged versions were constructed for these mutants and also for the UL11(CCC-) mutant (3) (Fig. 5). For all three viruses, the mutant proteins were expressed at levels that were even higher than wild-type UL11 (Fig. 8B), which was in part due to placement of the alleles in the U_L35 locus (3) (Fig. 5). As expected, mutant UL11(CCC-)-GFP exhibited normal gE packaging, like its untagged parent (Fig. 8A and C). In contrast, the other two GFP-tagged viruses exhibited different and surprising phenotypes. First, mutant UL11(Myr-)-GFP acquired the ability to package normal amounts of gE (Fig. 8A and C), even though this derivative is unable to bind membranes on its own (3). Together with the results of mutant UL11(CCC-), it appears that only small amounts of UL11 are needed for gE packaging, but in the absence of both fatty acid modifications, higher expression levels seem to be necessary for driving UL11 to where it is needed. Second, mutant UL11(AC-)-GFP remained incompetent for gE packaging (Fig. 8A and C) even though its UL11 derivative was expressed at high levels (Fig. 8B) and is capable of binding membranes (4). This finding indicates that the acid cluster of UL11 is critical for gE packaging. To rule out the possibility that the observed gE-packaging defect may stem from secondary mutations elsewhere, we analyzed a UL11(AC-)-GFP revertant, UL11-GFP, in which the acidic cluster was restored. The result revealed that the AC reversion completely restored the packaging of gE to wild-type level (data not shown), suggesting that the acidic cluster is the sole factor responsible for the observed defect.

The acidic cluster of UL11 is well known to be the major determinant for binding of UL16 (27), but it is also utilized by host factors for membrane trafficking when UL11 is expressed by itself (26). Acidic clusters from the Nef and furin proteins have been shown to substitute for that of UL11 with regard to the trafficking function (26), but these foreign sequences are not recognized by UL16 (28). To distinguish between these two functions in regard to the packaging of gE, recombinant viruses were constructed in which the UL11 acidic cluster was replaced with those of Nef or furin (Fig. 5). Both mutants

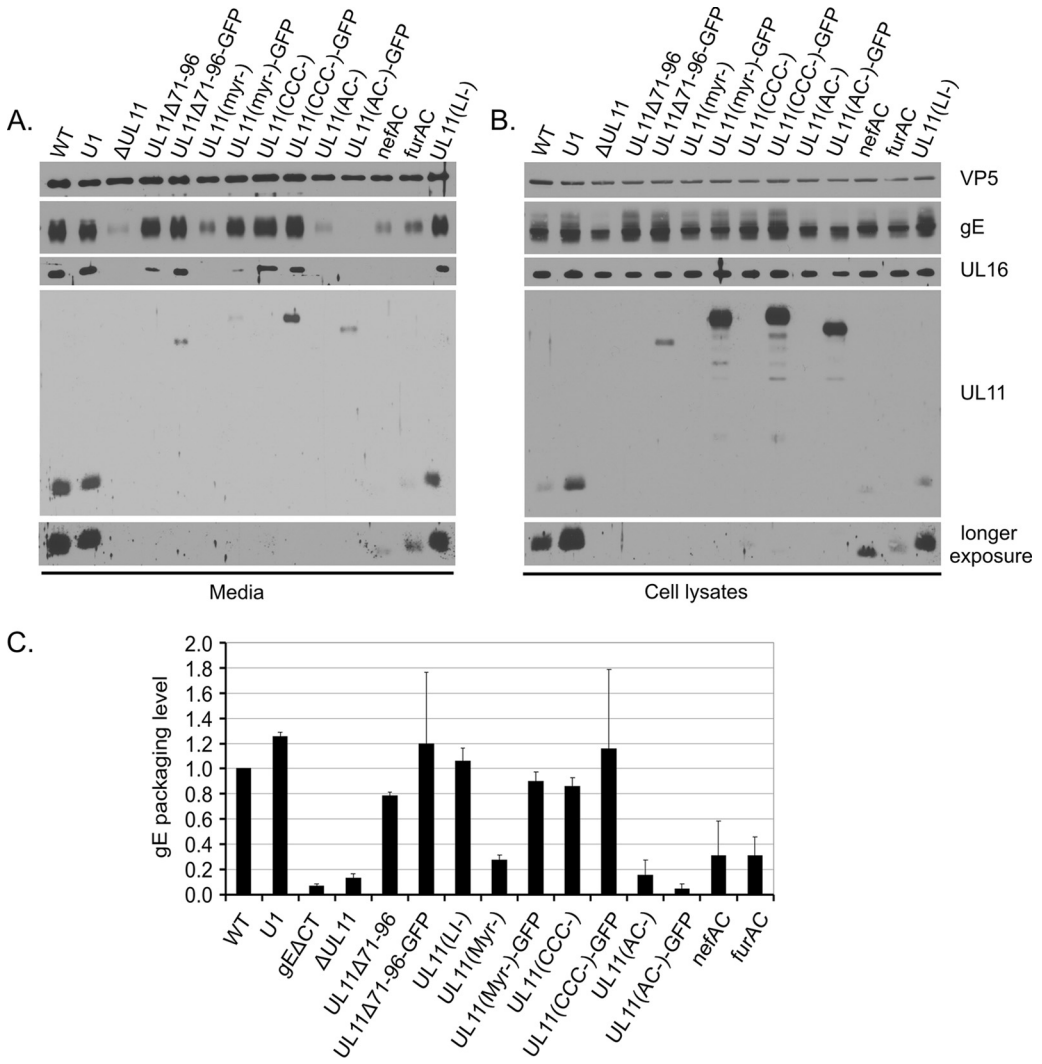

FIG. 8. Identification of the gE packaging determinant within UL11. (A) Wild type and UL11 mutants from infected-cell supernatants were pelleted through 30% sucrose cushions, and the virions were analyzed for the presence of VP5, gE, UL16, and UL11 by Western blotting. (B) Measurement of the intracellular expression levels of the corresponding proteins. (C) Quantitative analysis of gE in the UL11 mutant virions. The amount of gE in the various mutants was quantified and normalized to the level of wild-type gE. The data are representative of at least three independent experiments. WT, wild type.

formed small plaques comparable to UL11 AC-null (data not shown) and exhibited reduced growth kinetics (Fig. 6B). The UL11 chimeras were expressed at somewhat reduced levels (10 to 30%) relative to the wild type (Fig. 8B), but their packaging was reduced by >95% (Fig. 8A). With regard to gE, the foreign acidic clusters only partially restored packaging (10 to 20% increase), which is well below the wild-type level (Fig. 8A and C). This suggests the possibility that UL11 may work with UL16 for packaging gE into virions. Consistent with this, mutants lacking the native acidic cluster (ΔUL11, AC-, nefAC, and furAC) exhibited reduced packaging of UL16 (Fig. 8A), even though the expression levels were normal (Fig. 8B). Moreover, the gE species expressed by these mutants ran faster, indicating less extensive modifications of their carbohydrate side chains and limited movement through the secretory pathway. Thus, UL11 and UL16 may be needed to move gE to the site of budding rather than—or in addition to—providing bridging interactions with the capsid.

DISCUSSION

Although UL11 has been suggested to play an important role in HSV secondary envelopment, little is known about the underlying mechanism. Dissection of its interaction network represents a useful and meaningful approach to better understand the function of this protein during the HSV life cycle. The experiments described here strongly support the suggestion that UL11 and gE are in some sort of a complex, as suggested previously (16), but go much further by showing that the interaction is direct and by mapping the sequences involved in both proteins. Interestingly, the previous study (16) mapped the UL11-binding region to the C terminus of the gE tail

between the gE residues 495 to 550 in contrast to the residues 445 to 472 determined here. This discrepancy likely stems from different systems that were used for mapping the binding domain. The former was done under virus infection conditions, in which many other viral factors would compete with UL11 for binding to the gE tail, and could potentially confound the results. Also, we discovered that UL11 and gE are codependent for packaging. This finding was unexpected since both UL11 and gE have been thought to possess the necessary signals for trafficking to glycoprotein-enriched TGN, where the cytoplasmic tails of viral glycoproteins interact with tegument proteins to bridge tegument-coated capsids to membranes. Our data clearly suggest that, in the context of virus infection, something more is required. Thus, the virion packaging is not a merely passive, but rather a highly active and selective process, which is driven by specific protein-protein interactions.

The underlying mechanism of how the UL11-gE interaction facilitates mutual packaging is not clear; however, some clues may be extrapolated from the present study and work done in the past. We speculate that the observed dual packaging defects seen in the absence of either of the two proteins likely reflects a block in trafficking or complex formation. We hypothesize that UL11 and gE, together with UL16, need to form a complex before trafficking to the final budding compartment within TGN. The complex likely contains some other proteins such as UL21 and VP22, which in return may recruit cellular or viral factors that promote the trafficking to the budding niches. When either of the key components is missing, the transport complex will become loose or fall apart and a defect will arise. In support of this hypothesis, we noticed that the cell-associated gE exhibited less abundance in the slower migrating, mature form in the UL11-null and AC- mutants (Fig. 8). This suggests that UL11 may be needed for moving gE through the secretory pathway, where it becomes more heavily glycosylated on the way to the site of cytoplasmic budding.

The acidic cluster of UL11 appears to play an important role in regulating either complex formation or trafficking. Deletion or alteration of this motif resulted in a complete loss of function of UL11 in terms of growth kinetics (Fig. 6B) and plaque formation (data not shown). Stabilization of UL11(AC-) with GFP even had a negative effect on gE, UL16 packaging and viral growth rate despite the fact that UL11(AC)-GFP was well expressed (Fig. 6B) and retained the ability to bind gE as demonstrated by GST-pulldown assays (data not shown). Furthermore, replacement with foreign acidic clusters (nefAC or furAC) only partially rescued the viral growth curve, and the mutants were still defective for packaging UL11 and gE (Fig. 8A). One explanation is that foreign ACs direct UL11 to incorrect locations. The second explanation is that the authentic AC is required for recognition of UL16 (27), which may be part of the complex with gE and UL11. In support of this, we have shown that UL11 interacts with UL16 via its N terminus (49) and with gE via its C terminus (the present study). Thus, UL11 may serve as an adaptor protein to recruit UL16 to the gE complex, although it is clear from the data shown here that UL11 and gE can interact by themselves. Moreover, the companion paper (50) shows that UL16 also binds directly to the cytoplasmic domain of gE. Therefore, it is reasonable to assume that the three may form a tripartite complex during HSV infection. Further support for the involvement of UL16 is provided by its reduced packaging (~50% as shown in Fig. 7A and B) when the tail of gE was absent. Thus, a stable complex may be critical for individual protein trafficking and packaging besides that they may help recruit cellular sorting factors to promote trafficking.

VP22 may also be a part of the complex. As a binding partner (41, 45), VP22 is also critical for gE packaging as shown by a viral deletion mutant (13), although VP22 does not require gE for packaging of itself. Interestingly, instead of gE, we noticed that deletion of UL11 had some effect on VP22 in which the packaging of VP22 was reduced by 30 to 50% (Fig. 6B), suggesting that there may be some link between UL11 and VP22. Although there has been no report regarding whether UL11 or UL16 can interact with VP22, it is possible that they may work synergistically to promote the trafficking and packaging of the complex. Certainly, in addition to the abovementioned mechanisms, the gE packaging also involves a pathway that is independent of the gE tail since the gEΔCT mutant can still package certain amount of gE (Fig. 7). This is perhaps mediated via the interaction of the gE extracellular domain with gI and other glycoproteins.

Finally, the UL11-gE interaction and the proposed complex may have significance in gE-mediated cell-to-cell spread in addition to their important role in the secondary envelopment. Early in the infection, gE stays on nuclear membrane and around the TGN, but later it traffics to lateral cell junctions (34, 48). However, transfection of gE alone or cotransfection with gI does not lead to steady accumulation of gE on the cell surface (34), suggesting that other viral factors are likely involved in the gE transport. Mutants lacking the tail of gE tail behave just like the gE-null mutant in forming small plaques and being deficient in cell-to-cell spread (16), suggesting that the gE.CT is a driving force for gE-mediated lateral spread. Therefore, it is likely that the binding partners of the gE tail work in concert to assure the function of gE. Consistent with this hypothesis, UL11, UL16, and VP22-null viruses all form small plaques with sizes similar to as gEΔCT (13, 16). Moreover, VP22 has been shown to facilitate cell-to-cell spread *in vivo*. It has been proposed that gE on the virions may interact with receptors on cell surface at cell junctions (11, 42). If that is the case, efficient gE virion incorporation by UL11 will surely help HSV virion move across cell junctions.

In summary, we have described in detail the interaction that occurs directly between UL11 and the cytoplasmic tail of gE. We have identified a new function for UL11 in glycoprotein acquisition and another role for gE in tegumentation. The present study sheds light on the highly active and selective nature of herpesvirus secondary envelopment and may also have important implications for the role of UL11 in gE-mediated cell-to-cell spread.

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REFERENCES

1. **Baines, J. D., and B. Roizman.** 1991. The open reading frames UL3, UL4, UL10, and UL16 are dispensable for the replication of herpes simplex virus 1 in cell culture. J. Virol. **65:**938–944.
2. **Baines, J. D., and B. Roizman.** 1992. The UL11 gene of herpes simplex virus 1 encodes a function that facilitates nucleocapsid envelopment and egress from cells. J. Virol. **66:**5168–5174.
3. **Baird, N. L., J. L. Starkey, D. J. Hughes, and J. W. Wills.** 2010. Myristylation and palmitylation of HSV-1 UL11 are not essential for its function. Virology **397:**80–88.
4. **Baird, N. L., P. C. Yeh, R. J. Courtney, and J. W. Wills.** 2008. Sequences in the UL11 tegument protein of herpes simplex virus that control association with detergent-resistant membranes. Virology **374:**315–321.
5. **Basu, S., G. Dubin, M. Basu, V. Nguyen, and H. M. Friedman.** 1995. Characterization of regions of herpes simplex virus type 1 glycoprotein E involved in binding the Fc domain of monomeric IgG and in forming a complex with glycoprotein I. J. Immunol. **154:**260–267.
6. **Brack, A. R., J. M. Dijkstra, H. Granzow, B. G. Klupp, and T. C. Mettenleiter.** 1999. Inhibition of virion maturation by simultaneous deletion of glycoproteins E, I, and M of pseudorabies virus. J. Virol. **73:**5364–5372.
7. **Brack, A. R., et al.** 2000. Role of the cytoplasmic tail of pseudorabies virus glycoprotein E in virion formation. J. Virol. **74:**4004–4016.
8. **Brignati, M. J., J. S. Loomis, J. W. Wills, and R. J. Courtney.** 2003. Membrane association of VP22, a herpes simplex virus type 1 tegument protein. J. Virol. **77:**4888–4898.
9. **Dingwell, K. S., et al.** 1994. Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread in vivo and across junctions of cultured cells. J. Virol. **68:**834–845.
10. **Dingwell, K. S., L. C. Doering, and D. C. Johnson.** 1995. Glycoproteins E and I facilitate neuron-to-neuron spread of herpes simplex virus. J. Virol. **69:** 7087–7098.
11. **Dingwell, K. S., and D. C. Johnson.** 1998. The herpes simplex virus gE-gI complex facilitates cell-to-cell spread and binds to components of cell junctions. J. Virol. **72:**8933–8942.
12. **Dubin, G., et al.** 1994. Characterization of domains of herpes simplex virus type 1 glycoprotein E involved in Fc binding activity for immunoglobulin G aggregates. J. Virol. **68:**2478–2485.
13. **Duffy, C., et al.** 2006. Characterization of a UL49-null mutant: VP22 of herpes simplex virus type 1 facilitates viral spread in cultured cells and the mouse cornea. J. Virol. **80:**8664–8675.
14. **Elliott, G., G. Mouzakitis, and P. O'Hare.** 1995. VP16 interacts via its activation domain with VP22, a tegument protein of herpes simplex virus, and is relocated to a novel macromolecular assembly in coexpressing cells. J. Virol. **69:**7932–7941.
15. **Farnsworth, A., K. Goldsmith, and D. C. Johnson.** 2003. Herpes simplex virus glycoproteins gD and gE/gI serve essential but redundant functions during acquisition of the virion envelope in the cytoplasm. J. Virol. **77:**8481–8494.
16. **Farnsworth, A., T. W. Wisner, and D. C. Johnson.** 2007. Cytoplasmic residues of herpes simplex virus glycoprotein gE required for secondary envelopment and binding of tegument proteins VP22 and UL11 to gE and gD. J. Virol. **81:**319–331.
17. **Fulmer, P. A., J. M. Melancon, J. D. Baines, and K. G. Kousoulas.** 2007. UL20 protein functions precede and are required for the UL11 functions of herpes simplex virus type 1 cytoplasmic virion envelopment. J. Virol. **81:** 3097–3108.
18. **Gierasch, W. W., et al.** 2006. Construction and characterization of bacterial artificial chromosomes containing HSV-1 strains 17 and KOS. J. Virol. Methods **135:**197–206.
19. **Harper, A. L., et al.** 2010. Interaction domains of the UL16 and UL21 tegument proteins of herpes simplex virus. J. Virol. **84:**2963–2971.
20. **Hook, L. M., J. Huang, M. Jiang, R. Hodinka, and H. M. Friedman.** 2008. Blocking antibody access to neutralizing domains on glycoproteins involved in entry as a novel mechanism of immune evasion by herpes simplex virus type 1 glycoproteins C and E. J. Virol. **82:**6935–6941.
21. **Johnson, D. C., and J. D. Baines.** 2011. Herpesviruses remodel host membranes for virus egress. Nat. Rev. Microbiol. **9:**382–394.
22. **Johnson, D. C., M. C. Frame, M. W. Ligas, A. M. Cross, and N. D. Stow.** 1988. Herpes simplex virus immunoglobulin G Fc receptor activity depends on a complex of two viral glycoproteins, gE and gI. J. Virol. **62:**1347–1354.
23. **Kopp, M., H. Granzow, W. Fuchs, B. Klupp, and T. C. Mettenleiter.** 2004. Simultaneous deletion of pseudorabies virus tegument protein UL11 and glycoprotein M severely impairs secondary envelopment. J. Virol. **78:**3024–3034.
24. **Kopp, M., et al.** 2003. The pseudorabies virus UL11 protein is a virion component involved in secondary envelopment in the cytoplasm. J. Virol. **77:**5339–5351.
25. **Leege, T., et al.** 2009. Effects of simultaneous deletion of pUL11 and glycoprotein M on virion maturation of herpes simplex virus type 1. J. Virol. **83:**896–907.
26. **Loomis, J. S., J. B. Bowzard, R. J. Courtney, and J. W. Wills.** 2001. Intracellular trafficking of the UL11 tegument protein of herpes simplex virus type 1. J. Virol. **75:**12209–12219.
27. **Loomis, J. S., R. J. Courtney, and J. W. Wills.** 2003. Binding partners for the UL11 tegument protein of herpes simplex virus type 1. J. Virol. **77:**11417–11424.
28. **Loomis, J. S., R. J. Courtney, and J. W. Wills.** 2006. Packaging determinants in the UL11 tegument protein of herpes simplex virus type 1. J. Virol. **80:**10534–10541.
29. **Lubinski, J. M., H. M. Lazear, S. Awasthi, F. Wang, and H. M. Friedman.** 2011. The herpes simplex virus 1 IgG Fc receptor blocks antibody-mediated complement activation and antibody-dependent cellular cytotoxicity in vivo. J. Virol. **85:**3239–3249.
30. **MacLean, C. A., B. Clark, and D. J. McGeoch.** 1989. Gene UL11 of herpes simplex virus type 1 encodes a virion protein which is myristylated. J. Gen. Virol. **70:**3147–3157.
31. **MacLean, C. A., A. Dolan, F. E. Jamieson, and D. J. McGeoch.** 1992. The myristylated virion proteins of herpes simplex virus type 1: investigation of their role in the virus life cycle. J. Gen. Virol. **73:**539–547.
32. **McGraw, H. M., S. Awasthi, J. A. Wojcechowskyj, and H. M. Friedman.** 2009. Anterograde spread of herpes simplex virus type 1 requires glycoprotein E and glycoprotein I but not Us9. J. Virol. **83:**8315–8326.
33. **McGraw, H. M., and H. M. Friedman.** 2009. Herpes simplex virus type 1 glycoprotein E mediates retrograde spread from epithelial cells to neurites. J. Virol. **83:**4791–4799.
34. **McMillan, T. N., and D. C. Johnson.** 2001. Cytoplasmic domain of herpes simplex virus gE causes accumulation in the *trans*-Golgi network, a site of virus envelopment and sorting of virions to cell junctions. J. Virol. **75:**1928–1940.
35. **Meckes, D. G. J., J. A. Marsh, and J. W. Wills.** 2010. Complex mechanisms for the packaging of the UL16 tegument protein into herpes simplex virus. Virology **398:**208–213.
36. **Meckes, D. G. J., and J. W. Wills.** 2007. Dynamic interactions of the UL16 tegument protein with the capsid of herpes simplex virus. J. Virol. **81:**13028–13036.
37. **Mettenleiter, T. C.** 2002. Herpesvirus assembly and egress. J. Virol. **76:**1537–1547.
38. **Mettenleiter, T. C., B. G. Klupp, and H. Granzow.** 2009. Herpesvirus assembly: an update. Virus Res. **143:**222–234.
39. **Nagashunmugam, T., et al.** 1998. In vivo immune evasion mediated by the herpes simplex virus type 1 immunoglobulin G Fc receptor. J. Virol. **72:** 5351–5359.
40. **O'Regan, K. J., M. J. Brignati, M. A. Murphy, M. A. Bucks, and R. J. Courtney.** 2010. Virion incorporation of the herpes simplex virus type 1 tegument protein VP22 is facilitated by *trans*-Golgi network localization and is independent of interaction with glycoprotein E. Virology **405:**176–192.
41. **O'Regan, K. J., M. A. Bucks, M. A. Murphy, J. W. Wills, and R. J. Courtney.** 2007. A conserved region of the herpes simplex virus type 1 tegument protein VP22 facilitates interaction with the cytoplasmic tail of glycoprotein E (gE). Virology **358:**192–200.
42. **Polcicova, K., K. Goldsmith, B. L. Rainish, T. W. Wisner, and D. C. Johnson.** 2005. The extracellular domain of herpes simplex virus gE is indispensable for efficient cell-to-cell spread: evidence for gE/gI receptors. J. Virol. **79:** 11990–12001.
43. **Saldanha, C. E., et al.** 2000. Herpes simplex virus type 1 glycoprotein E domains involved in virus spread and disease. J. Virol. **74:**6712–6719.
44. **Snyder, A., K. Polcicova, and D. C. Johnson.** 2008. Herpes simplex virus gE/gI and US9 proteins promote transport of both capsids and virion glycoproteins in neuronal axons. J. Virol. **82:**10613–10624.
45. **Stylianou, J., K. Maringer, R. Cook, E. Bernard, and G. Elliott.** 2009. Virion incorporation of the herpes simplex virus type 1 tegument protein VP22 occurs via glycoprotein E-specific recruitment to the late secretory pathway. J. Virol. **83:**5204–5218.
46. **Wang, F., et al.** 2010. Herpes simplex virus type 2 glycoprotein E is required for efficient virus spread from epithelial cells to neurons and for targeting viral proteins from the neuron cell body into axons. Virology **405:**269–279.
47. **Wills, J. W., R. C. Craven, and J. A. Achacoso.** 1989. Creation and expression of myristylated forms of Rous sarcoma virus Gag protein in mammalian cells. J. Virol. **63:**4331–4343.
48. **Wisner, T. W., and D. C. Johnson.** 2004. Redistribution of cellular and herpes simplex virus proteins from the *trans*-Golgi network to cell junctions without enveloped capsids. J. Virol. **78:**11519–11535.
49. **Yeh, P.-C., D. G. J. Meckes, and J. W. Wills.** 2008. Analysis of the interaction between the UL11 and UL16 tegument proteins of herpes simplex virus. J. Virol. **82:**10693–10700.
50. **Yeh, P.-C., et al.** 2011. Direct and specific binding of the UL11 tegument protein of herpes simplex virus to the cytoplasmic tail of glycoprotein E. J. Virol. **85:**9425–9436.